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TITLE: Neuroinflammatory Pathobiology in Gulf War Illness: Characterization with an Animal Model

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14. ABSTRACT
Gulf War Illness (GWI) is a multi-symptom syndrome with features of an inflammatory response due to infection or injury, findings suggestive of a chronic neuroimmune/neuroinflammatory disorder. *Our overarching hypothesis is that exposure to GWI-relevant compounds lead to enhanced and/or prolonged expression of proinflammatory mediators in the brain.* Our overall objective is to establish a neuroinflammatory model of GWI-related exposures, to define the contribution of high physiological stress, and to assess the potential for pharmacotherapy to ameliorate these effects. Work to date has established dosing regimens for the toxicant exposures and has begun to examine the time course of the neuroinflammatory response. The combination of chronic high stress levels and/or cholinesterase inhibitors greatly augmented the neuroinflammatory response to a nerve gas surrogate in multiple brain regions. These observations suggest a possible critical and unrecognized interaction between the stressful environs of the Gulf War theater and agent exposures unique to this conflict.

15. SUBJECT TERMS
Gulf War illness, chronic neuroinflammation, diisopropyl phosphorofluoridate, physiological stress, minocycline, cytokines, hippocampus

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Introduction

Gulf War Illness (GWI) is a multi-symptom disorder with features characteristic of "sickness" behavior including, cognitive impairment, fatigue, depression, sleep disruption, gastrointestinal and dermatological problems. Sickness behavior, a normal manifestation of an inflammatory response due to infection or injury, resolves when homeostasis is restored but in GWI the symptoms persist, findings suggestive of a heightened or chronic neuroimmune/neuroinflammatory disorder. The expression of proinflammatory cytokines and chemokines are the basis of sickness behavior and are the key elements of inflammation. Notably, low level inhalation exposure of experimental animals to sarin, the nerve agent implicated in GWI, causes neuroinflammation; further, the sarin surrogate diisopropyl phosphorofluoridate (DFP), increases proinflammatory cytokine expression in multiple brain regions. Finally, the stress of the war theater may have affected the blood brain barrier, allowing GWI-relevant agents access to the CNS. A role for stress in GWI is bolstered by our finding of increased proinflammatory cytokine expression in brain when DFP was preceded by a week of treatment with the rodent specific stress hormone, corticosterone (CORT). The above suggest a possible critical and unrecognized link between the stressful environment of the Gulf War (GW) theater, agent exposure(s) unique to this war, and a resulting adverse heightened neuroinflammatory outcome. *Our overarching hypothesis is that exposure to GWI-relevant compounds lead to enhanced and/or prolonged expression of proinflammatory mediators in the brain*. Our data also lead us to hypothesize that brief high physiological levels of the stress hormone, cortisol (CORT in the rodent) greatly exacerbate the effects of GWI-related exposures and that the FDA-approved anti-inflammatory, minocycline, can ameliorate heightened neuroinflammation. *Thus, our overall objective is to establish a neuroinflammatory model of GWI-related exposures, to define the contribution of high physiological stress in the initiation, strength and duration of the neuroinflammation observed and to assess the potential for currently available pharmacotherapy to ameliorate these effects with the ultimate goal of treating veterans suffering from GWI.*

Body

The accomplishments associated with the first year of the project are outlined below with linkage to each task in the detailed Statement of Work.

Timeline: Because work at the Centers for Disease Control and Prevention could not be initiated until receipt of the DOD-initiated and UIC-approved CRADA, funds were not available and work on the Project did not commence until Oct. 1, 2010, the beginning of the fiscal year (after late July 2010 approval of the CRADA between UIC and CDC). Thus, the progress reported to date has occurred during the first 11 months of the project.

Task 1: **Obtain Institutional Animal Care and Use Committee approval for use of the proposed number of animals (1240 mice) to be used in the Project.** IAACUC Approvals were obtained at both institutions.

Task 2: **Conduct dose ranging studies for the compounds to be evaluated alone and in combination (i.e. pyridostigmine bromide (PB), diethyl-m-toluamide (DEET),**

lipopolysaccharide (LPS), corticosterone (CORT) and diisopropyl phosphorofluoridate (DFP) . Dose-ranging experiments established the following well-tolerated regimens: PB (2 mg/kg/day, s.c.), DEET (30 mg/kg/day, s.c.) for 14 days; CORT (200μg/ml drinking water) on days 7-14 and DFP (4mg/kg, i.p) or LPS (2 mg/kg, s.c.) administered as single dose on day 15.

Tasks 3, 4 and 5: Determine duration of the neuroinflammation caused by CORT & LPS and CORT & DFP with and without co-treatment with PB & DEET and with potential protection by the anti-inflammatory agent, minocycline (MINO) . The 15 day time point has been completed for not only CORT and LPS but also in combination with PB & DEET and MINO. Tissue has been prepared and analyzed for proinflammatory cytokines and chemokines following all treatments except MINO. MINO data will be available in mid September (11.5 month of the project). We found that DFP alone (Figures 2 and 4), as well as LPS alone (Figures 1 and 3), caused a marked neuroinflammation as assessed by qRT-PCR of IL-1β, TNF-α, IL-6, chemokine (C-C motif) ligand 2 (CCL-2), leukemia inhibitory factor (LIF), oncostatin-M (OSM) and IL-10. Chronic pretreatment with high physiological levels of CORT and/or PB and DEET greatly augmented (up to 500-fold) the neuroinflammatory response to LPS (Figures 1 and 3) and DFP (Figures 2 and 4) in hippocampus (Figures 3 and 4), cortex (Figures 1 and 2) and striatum (data not shown). These findings have been summarized in Abstract form and will be presented at the Annual Meeting for the Society for Neuroscience in Washington, D.C. in November 2011 (see below).

Task 8: The efforts to provide functional correlates to the neuroinflammation resulting from various combinations of the cholinesterase inhibitors, chronic stressors, and inflammagens by the implementation of synaptic plasticity paradigms were proposed for years 2 and 3 of the project. These studies are being initiated at the University of Illinois College of Medicine – a postdoctoral associate has been appointed and this work will begin shortly.

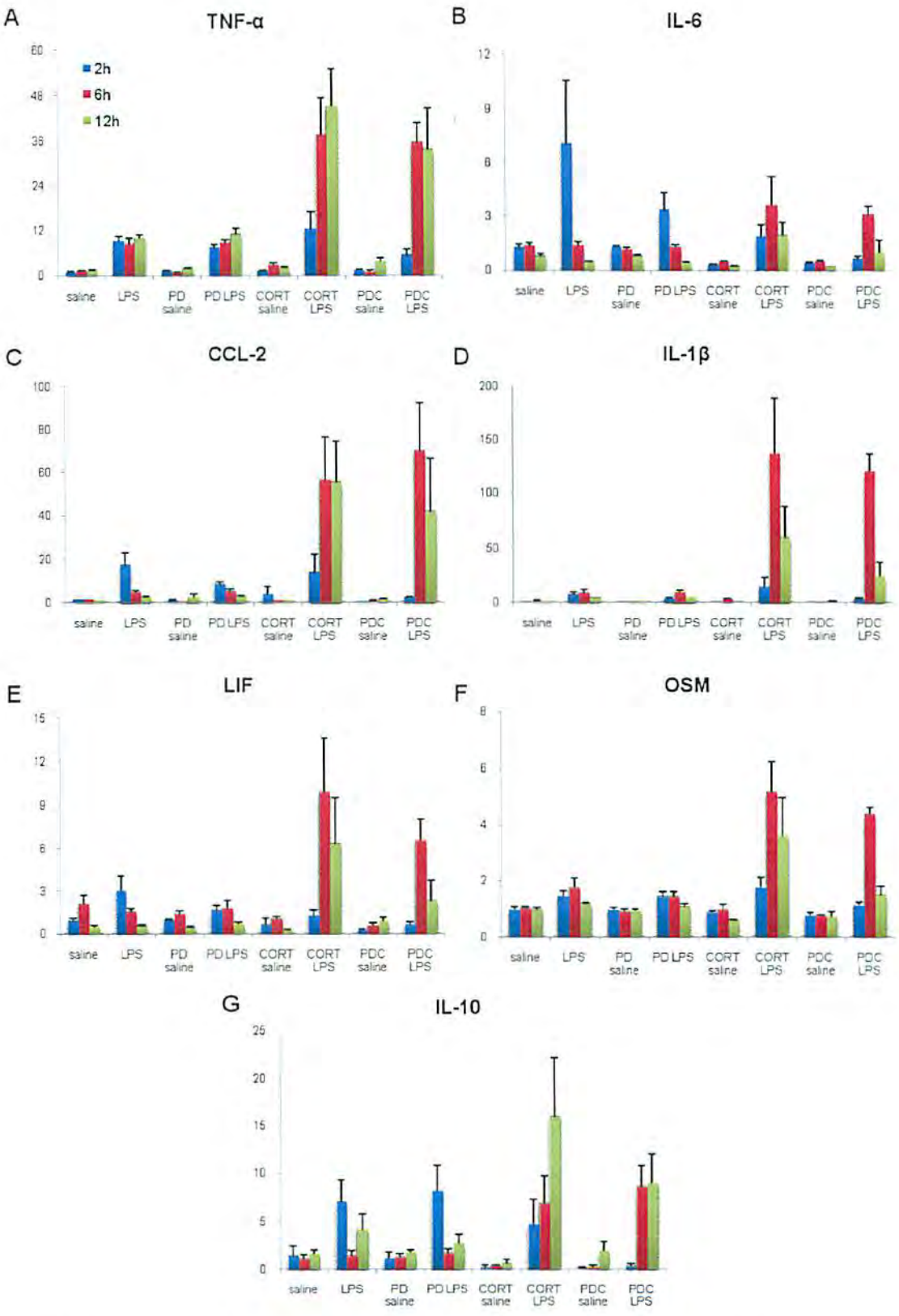

Figure 1: Effects of Chronic CORT and LPS on proinflammatory cytokines in the cortex at 2, 6 and 12 hours after LPS exposure. Mice were treated with PB (2 mg/kg/day, s.c.) and DEET (30 mg/kg/day, s.c.) denoted in the figure as PD for 14 days; CORT (200 µg/ml drinking water) on days 7-14 denoted as either CORT or C when administered with PB and DEET (PDC); and LPS (2 mg/kg, s.c.) as a single dose on day 15. At 2, 6 or 12 h post LPS exposure, mice were sacrificed and total RNA was extracted from the cortex. Real-time PCR analysis was performed for TNF-α (A), IL-6 (B), CCL-2 (C), IL-1β (D), LIF (E), OSM (F) and IL-10 (G). Bars represent mean ± SEM (N=5 mice/group).

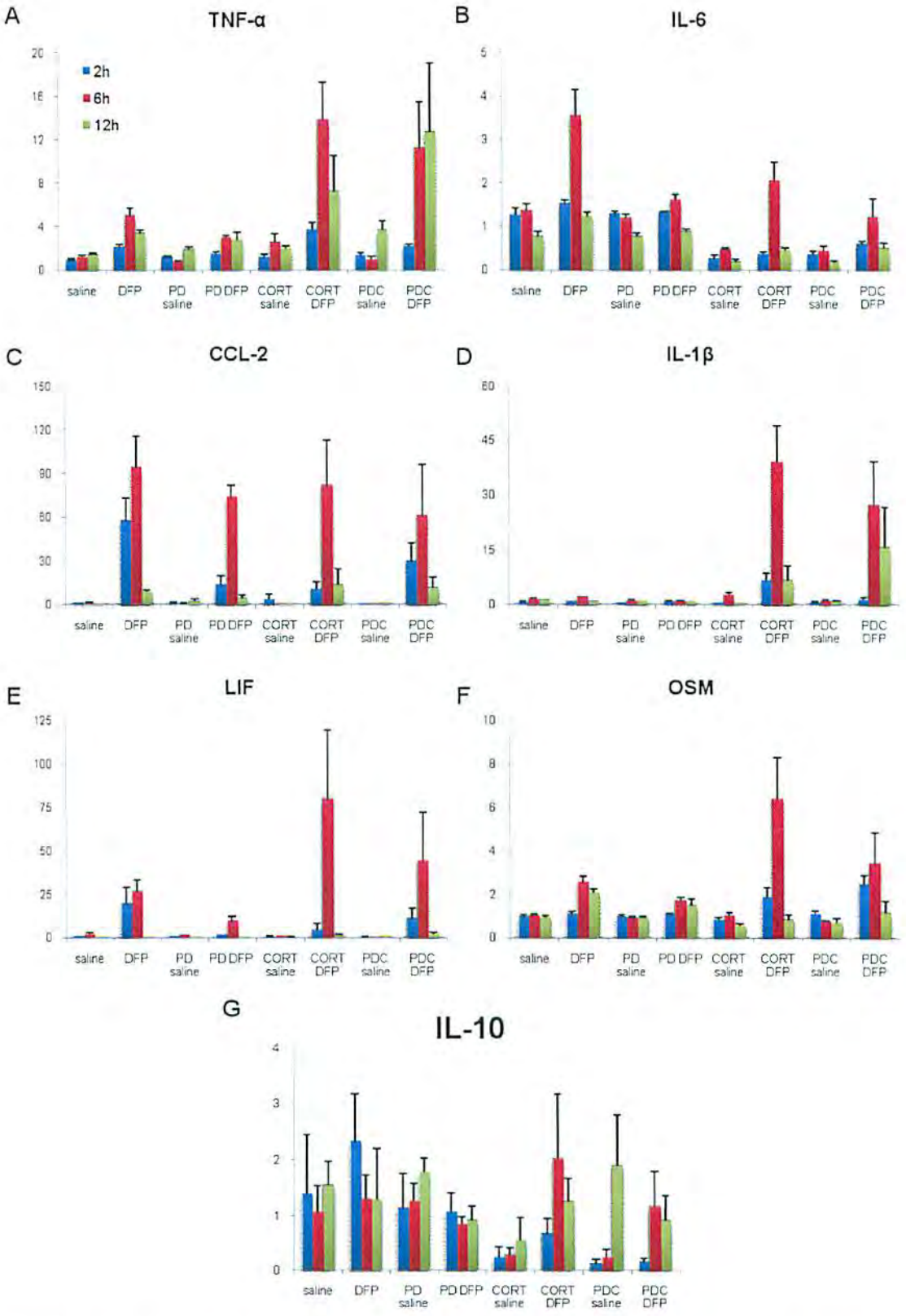

Figure 2: Effects of Chronic CORT and DFP on proinflammatory cytokines in the cortex at 2, 6 and 12 hours after DFP exposure. Mice were treated with PB (2 mg/kg/day, s.c.) and DEET (30 mg/kg/day, s.c.) denoted in the figure as PD for 14 days; CORT (200 µg/ml drinking water) on days 7-14 denoted as either CORT or C when administered with PB and DEET (PDC); and DFP (4 mg/kg, i.p.) as a single dose on day 15. At 2, 6 or 12 h post DFP exposure, mice were sacrificed and total RNA was extracted from the cortex. Real-time PCR analysis was performed for TNF-α (A), IL-6 (B), CCL-2 (C), IL-1β (D), LIF (E), OSM (F) and IL-10 (G). Bars represent mean ± SEM (N=5 mice/group).

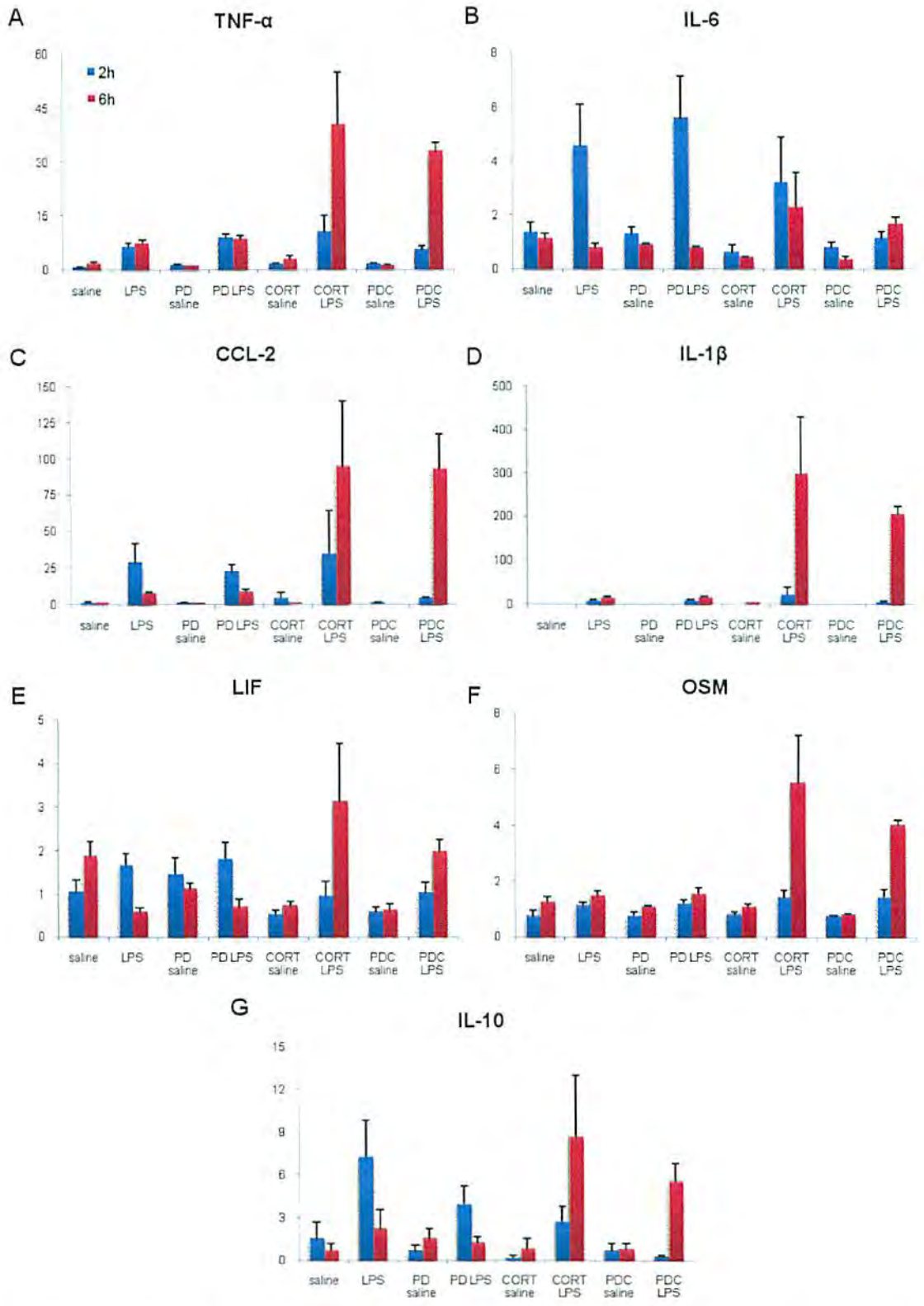

Figure 3: Effects of Chronic CORT and LPS on proinflammatory cytokines in the hippocampus at 2 and 6 hours after LPS exposure. Mice were treated with PB (2 mg/kg/day, s.c.) and DEET (30 mg/kg/day, s.c.) denoted in the figure as PD for 14 days; CORT (200 µg/ml drinking water) on days 7-14 denoted as either CORT or C when administered with PB and DEET (PDC); and LPS (2 mg/kg, s.c.) as a single dose on day 15. At 2 and 6 h post LPS exposure, mice were sacrificed and total RNA was extracted from the hippocampus. Real-time PCR analysis was performed for TNF-α (A), IL-6 (B), CCL-2 (C), IL-1β (D), LIF (E), OSM (F) and IL-10 (G). Bars represent mean ± SEM (N=5 mice/group).

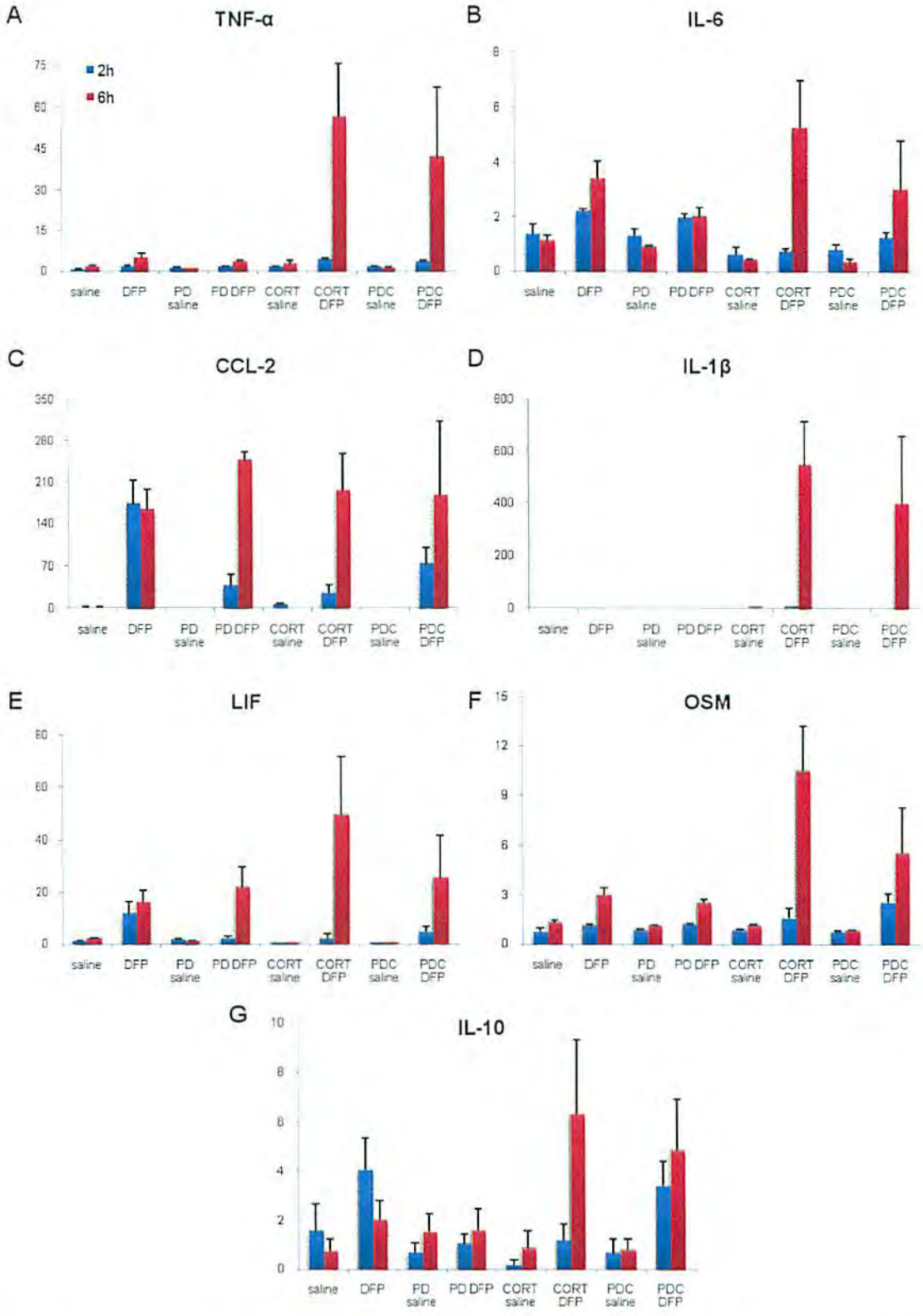

Figure 4: Effects of Chronic CORT and DFP on proinflammatory cytokines in the hippocampus at 2 and 6 hours after DFP exposure. Mice were treated with PB (2 mg/kg/day, s.c.) and DEET (30 mg/kg/day, s.c.) denoted in the figure as PD for 14 days; CORT (200 µg/ml drinking water) on days 7-14 denoted as either CORT or C when administered with PB and DEET (PDC); and DFP (4 mg/kg, i.p.) as a single dose on day 15. At 2 and 6 h post DFP exposure, mice were sacrificed and total RNA was extracted from the hippocampus. Real-time PCR analysis was performed for TNF-α (A), IL-6 (B), CCL-2 (C), IL-1β (D), LIF (E), OSM (F) and IL-10 (G). Bars represent mean ± SEM (N=5 mice/group).

Key Research Accomplishments

1. Demonstration that the nerve agent, DFP is proinflammatory in the nervous system of mice
2. Demonstration that prior administration of high physiological stress levels of corticosterone markedly augment the neuroinflammatory effect of DFP and the known inflammogen, LPS
3. Demonstration that the concomitant administration of PB and DEET do not augment the proinflammatory response to DFP or LPS.

Reportable Outcomes

2011 Society for Neuroscience Abstract: Chronic exposure to glucocorticoids, PB and DEET primes the neuroinflammatory response to the nerve agent DFP in a potential model for Gulf War Illness.K.A. Kelly[1]; D.B. Miller[1]; S.M. Lasley[2]; J.P. O'Callaghan[1], 1. CDC-NIOSH, Morgantown, WV; 2. University of Illinois College of Medicine, Peoria, IL.

This abstract was selected by the Society for Neuroscience as being newsworthy. A lay version of the abstract's content and significance of the research will be available to the press during the Annual Meeting in November.

Conclusions to date:

Our findings are suggestive of a possible critical and as yet unrecognized interaction between the stressful environs of the GW theater and agent exposure(s) unique to this war, exposures in which the CNS is primed to amplify future exposures to pathogens, injury or toxicity. Such occurrences could potentially result in prolonged episodes of sickness behavior.

Appendices

1: Task Summary Table
2: Society for Neuroscience abstract

#1

CDMRP task completed summary as of 9/6/11

Date	Sacrifice Time Point	Time after LPS or DFP	MINO Pretreat-ment	Brain area dissected	Collection of Serum, Liver	q-RT PCR end points assayed*	GFAP ELISA
2/24/2011	15 day	2 hr	-	Hip, Ctx,Str, Cb OB, Hypoth	X	OSM,GFAP,IL6,CCL2, TNF, LI1B, Lif, IL10	
2/22/2011	15 day	6 hr	-	Hip, Ctx,Str, Cb OB, Hypoth	X	OSM,GFAP,IL6,CCL2, TNF, LI1B, Lif, IL10	
4/5/2011	15 day	12 hr	-	Hip, Ctx,Str, Cb OB, Hypoth	X	OSM,GFAP,IL6,CCL2, TNF, LI1B, Lif, IL10	
4/7/2011	15 day	72 hr	-	Hip, Ctx,Str, Cb OB, Hypoth	X	-	Hip, Ctx, Str
8/22/2011	15 day	6 hr	X	Hip, Ctx,Str, Cb OB, Hypoth	X	-	
8/25/2011	15 day	12 hr	X	Hip, Ctx,Str, Cb OB, Hypoth	X	-	

Hip;Hippocampus, Ctx;Cortex, Str;Striatum, Cb;Cerebellum, OB;Olfactory Bulb, Hypoth;Hypothalamus
*PCR assay limited to Hippocampus, Cortex as of 9/6/11

#2

Chronic exposure to glucocorticoids, PB and DEET primes the neuroinflammatory response to the nerve agent DFP in a potential model for Gulf War Illness.

K.A. Kelly[1]; D.B. Miller[1]; S.M. Lasley[2]; J.P. O'Callaghan[1], 1. CDC-NIOSH, Morgantown, WV; 2. University of Illinois College of Medicine, Peoria, IL.

We have shown that chronic exposure to the glucocorticoid, corticosterone (CORT), at levels associated with high physiological stress, can prime the CNS proinflammatory response to neurotoxic exposures and systemic inflammation. Such neuroinflammatory events can be associated with sickness behavior. Gulf War (GW) Illness is a multi-symptom disorder with features characteristic of persistent sickness behavior. GW veterans were exposed to the stresses of war, prophylactic treatment with the reversible acetylcholinesterase (AChE) inhibitor pyridostigmine bromide (PB), the insect repellent DEET and, potentially, the nerve agent, sarin. These combined exposures were designed to mimic the conditions existing in theater during the 1991 GW. Here we examined whether CORT exposures primed the CNS to mount neuroinflammatory responses to GW exposures. Male C57BL/6 mice were treated with chronic (14 days) PB (2 mg/kg/day, s.c.) and DEET (30 mg/kg/day, s.c.); as well as subchronic exposure to CORT (200 μg/ml in the drinking water on days 7-14) followed by acute exposure (day 15) to the sarin surrogate and irreversible AChE inhibitor, diisopropyl phosphorofluoridate; (DFP) (4 mg/kg, i.p.) or known inflammogen, lipopolysaccharide (LPS) (2 mg/kg, s.c.). We found that DFP alone, as well as LPS alone, caused a marked neuroinflammation as assessed by qRT-PCR of IL1β, TNFα, IL6, CCL2, LIF and OSM. Chronic pretreatment with high physiological levels of CORT and/or PB and DEET greatly augmented (up to 500-fold) the neuroinflammatory response to LPS and DFP in hippocampus, cortex and striatum. Our findings are suggestive of a possible critical and as yet unrecognized interaction between the stressful environs of the GW theater and agent exposure(s) unique to this war, exposures in which the CNS is primed to amplify future exposures to pathogens, injury or toxicity. Such occurrences could potentially result in prolonged episodes of sickness behavior. (Supported by CDMRP)